Arresting serotonin

Atheir Abbas* and Bryan L. Roth†‡

**Department of Biochemistry, Case Western Reserve University School of Medicine, Cleveland, OH 44106; and †Departments of Pharmacology and Medicinal Chemistry, and the National Institute of Mental Health Psychoactive Drug Screening Program, University of North Carolina, Chapel Hill, NC 27599*

After decades of relative quiescence, G protein coupled receptor theory (GPCR) is undergoing a conceptual revolution driven in large part by findings like those of Schmid *et al.* as reported in this issue of PNAS (1). Since 1966, hypotheses of drug action at GPCRs have been driven by the quaint notion of "intrinsic efficacy" (2), which proposes that drugs which completely activate GPCRs (e.g., full agonists) remain full agonists regardless of the cellular milieu (see ref. 3 for a recent review). In other words, to paraphrase Gertrude Stein, "an agonist is an agonist is an agonist." Over the past several years, based mainly on *in vitro* findings with synthetic agonists, it has become evident that the notion of "intrinsic efficacy" is a myth and that the cellular milieu is a critical determinant of drug action. As Schmid *et al.* elegantly demonstrate, the actions *in vivo* of the naturally occurring agonist serotonin (5-hydroxytryptamine; 5-HT) are profoundly altered by the complement of arrestins expressed in neurons (1). These findings will force neuropharmacologists to fundamentally alter their notions of drug actions at neuronal receptors and will have a major impact on central nervous system (CNS) drug discovery efforts.

For many years it has been clear that the hallucinogens lysergic acid diethylamide (LSD), psilocybin, and mescaline exert their actions *in vivo* principally via 5-HT_{2A} serotonin receptor activation (4). According to classical concepts of receptor pharmacology, one would predict any drug that activates 5-HT_{2A} serotonin receptors will be hallucinogenic. It has also been widely appreciated for decades, however, that several small molecules, including the endogenous agonist 5-HT, can activate 5-HT_{2A} receptors but do not induce hallucinations. Thus, for instance, the LSD analogue lisuride, which is prescribed in Europe for the treatment of Parkinson's disease, is a potent 5-HT_{2A} agonist devoid of appreciable hallucinogenic actions *in vivo* (4, 5). Additionally, several groups have demonstrated that various 5-HT_{2A} agonists cause differential patterns of signal transduction *in vitro* and *in vivo* (5–7). Invariably, when a large number of 5-HT_{2A} receptor agonists are examined, discrete patterns of signal transduction are seen. Thus, for instance, the hallucinogenic agonists 2,5-dimethoxy-4-iodoamphetamine (DOI) and LSD and the nonhallucinogenic agonists lisuride and 5-HT all evoke distinctive patterns of activation of phospholipase C, arachidonic acid release, and transcription factor expression in cells expressing 5-HT_{2A} receptors (5–7).

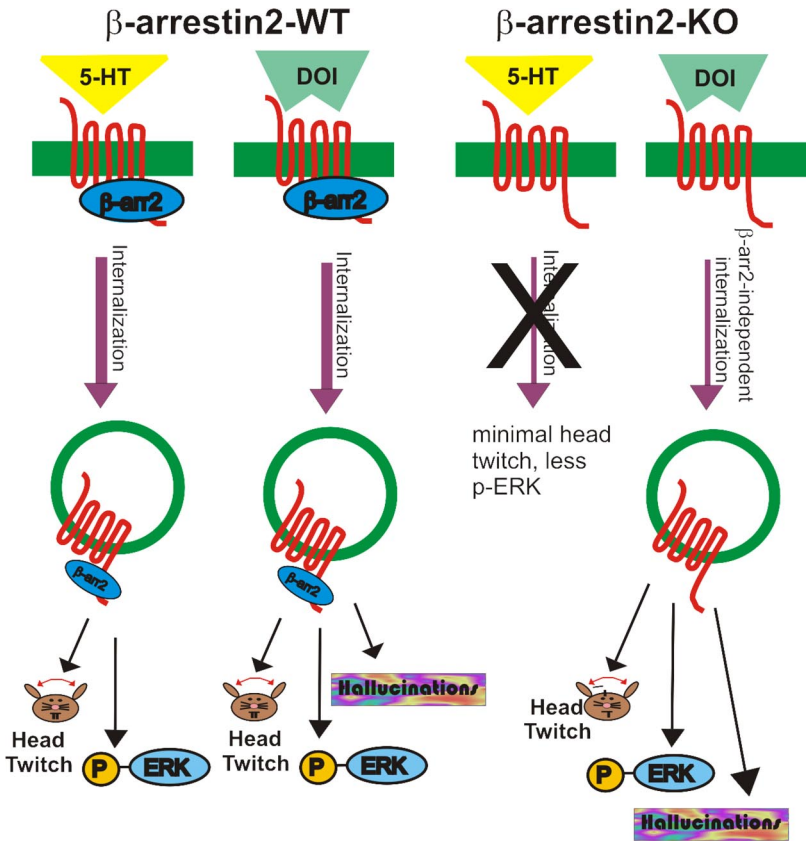

Fig. 1. Functional selectivity at the 5-HT_{2A} receptor is mediated by β-arrestin. The absence of β-arrestin2 abrogates many 5-HT induced downstream events at 5-HT_{2A} receptors, including internalization, head twitch, and *p*-ERK, but has little to no effect on those same signaling pathways when DOI is the ligand in question.

A number of researchers have attempted to modify the framework underlying receptor theory to accommodate the aforementioned phenomena, and many of the ideas generated fall under the rubric of "functional selectivity," "biased agonism," "agonist directed trafficking of receptor stimulus," and so on (8). In this review, we will use the term functional selectivity (3). There are three key types of observations which support the concept of functional selectivity. First, it is clear that there are multiple active states for GPCRs and that receptor populations exist in what are essentially conformational assemblages (8). Second, different ligands either induce or stabilize different ensembles of active conformations (3). Similarly, networks of GPCR-interacting proteins, including arrestins, G proteins, and caveolins (to name just a few), form unique local environments, leading to differential patterns of agonist responsiveness (9, 10). Third, these different active states lead to the different signaling pathways downstream of a single GPCR. Thus, ligands can selectively activate a subset of downstream signaling

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See companion article on page 1079.

‡To whom correspondence should be addressed. E-mail: bryan_roth@med.unc.edu.

pathways by inducing or stabilizing the appropriate active states.

Schmid *et al.* present a compelling set of observations that can only be explicated by the emerging concept of functional selectivity (1). The authors begin by examining the head twitch response, a 5-HT_{2A} receptor activation-mediated behavior characteristic of hallucinogens (11), in wild-type and β-arrestin2 knockout mice. The use of β-arrestin2 knockout mice was fortuitous because of an emerging body of data indicating that GPCRs use arrestins for promoting an "arrestinergic" pattern of signaling (reviewed in ref. 9). Schmid *et al.* find that injections of the 5-HT precursor 5-hydroxytryptophan (5-HTP) induce a robust head-twitch response in wild-type, but not in β-arrestin2 knockout, mice. Amazingly, the synthetic hallucinogen DOI elicits the same robust response in wild-type and β-arrestin2 knockout mice. The authors also demonstrate that deleting β-arrestin2 does not alter 5-HT_{2A} receptor levels and that both responses are blocked by a selective 5-HT_{2A} antagonist. These findings indicate that the *in vivo* responses to the agonists 5-HT and DOI at a single GPCR are differentially altered by the presence of β-arrestin2 scaffold.

Schmid *et al.* then perform an elegant series of *in vitro* and *in vivo* biochemical and cell biological studies to elucidate the potential mechanisms responsible for this differential responsiveness. The authors show, for example, a differential pattern of β-arrestin sensitivity for agonist-mediated internalization and ERK phosphorylation in murine embryonic fibroblasts and cortical pyramidal cells in culture (see Fig. 1). In agreement with prior studies, they demonstrate that the β-arrestin sensitivity is critically dependent upon the cellular milieu (12). They also demonstrate that the differential arrestin-requirement recapitulates, in some but not all instances, the differential arrestin requirement for *in vivo* behavioral responses.

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Altogether, the findings of Schmid *et al.* cannot be reconciled with classical pharmacology theory. For example, the initial finding that 5-HTP-induced head twitch is dramatically reduced in β-arrestin2 knockout mice leads to the prediction that DOI's effects must be similarly diminished. Instead, deleting β-arrestin2 differentially alters the response to 5-HTP and DOI. The same holds true for the distinctively differential effects of arrestin depletion on signaling induced by DOI and 5-HT. However, if one considers the possibility that 5-HT and DOI induce (or stabilize) discrete ensembles of active conformations, the above data can be readily explained by functional selectivity (see Fig. 1). Indeed, the findings by Schmid *et al.* suggest that the absence of β-arrestin has a far more dramatic effect in terms of changing the conformational ensembles that can be induced or stabilized by 5-HT as compared with DOI. Lefkowitz and colleagues (9, 17, 18) have also suggested that arrestins may differentially modulate the actions of GPCR agonists.

The studies by Schmid *et al.*, as well as others (13), also have implications for therapeutic drug discovery. Given that ligands apparently induce differential patterns of signal transduction, it should be possible to design drugs that stabilize these unique conformational ensembles. This theoretical framework may be helpful in designing useful drugs by helping to determine what sort of stimulus pathway activation profile is most therapeutic, and, conversely, which pathways might be responsible for undesirable side effects. Indeed, we and others have suggested that novel atypical antipsychotic drugs that activate D2-dopamine receptors (e.g., aripiprazole, bifeprunox, preclamol) are beneficial precisely because of their functional selectivity (14, 15). One can envision a scenario whereby drugs are identified via differential actions at a panel of functional readouts, yielding compounds with enhanced therapeutic efficacy and fewer side effects (16). Given the multiplicity of signaling events that can be elicited by a single GPCR, it is likely that researchers in academia and the pharmaceutical industry will be exploiting the predictions that result from the functional selectivity hypothesis for years to come.

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